VERIFICATION OF TRANSLATION

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Fraunhofer-Gesellschaft zur Förderung der angewandten Forschung eV

I, the undersigned, am conversant with both the German and English languages and I hereby declare that the attached is a true and faithful English translation of the German priority document specified above.

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The attached papers are a true and exact copy of the originally filed documents of this patent application.

Munich, the 11th day of September 2003
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Forisomes, Method for Their Isolation, and Their Use as a Molecular Working Machine

The present invention relates to forisomes, i.e., protein bodies which are also referred to as crystalline P-proteins, and which, according to the present knowledge, are present exclusively, but ubiquitously, in plants of the legume family (*Fabaceae*, *Papillionaceae*). These protein bodies have very extraordinary, heretofore unknown, properties which enable their use as molecular working machines. Moreover, the invention relates to a method for isolating forisomes from their natural environment.

The phloem of higher plants contains a system of microtubules (sieve elements) extending throughout the plant and serving for transporting photo assimilates (see Figure 2). The sieve elements are comprised of cells that, as a whole, form a capillary system through the entire plant, i.e., the aforementioned phloem. This is a micro fluidic system having an inner pressure of up to 3.5 MPa and maximum flow velocities up to 3 cm/min^{-1}.

Crystalline P-proteins are specific ingredient bodies of the sieve elements of the legume family (*Fabaceae*). They were first observed in plant cuts and described as usually elongate compact structures up to a length of 30 µm with a highly ordered ("crystalloid") ultra structure. Their function, however, remained unclear for more than a century after their discovery (Behnke, H.D., Nondispersive protein bodies in sieve elements: a survey and review of their origin, distribution and taxonomic significance. IAWA Bull, 12, 143-175 (1991)). In the meantime, it was found that they deform suddenly upon the occurrence of drastic changes of the hydrostatic pressure in the interior of the sieve elements. The proteins convert from an ordered "crystalloid" state (spindle shaped) (see reference numeral 2 in Figure 2) into a "disperse" conformation that ultra-structurally appears to be less ordered (see reference numeral 1 in Figure 2; in this Figure 3 describes a sieve plate and 4 the sieve elements). The disperse form has a rounded configuration and forms plugs in the sieve tubes and reduces thus the further flow of the liquid rich with photo assimilates within the tube system. However, in this connection the protein bodies apparently do not lose their internal organization completely because they

are still capable of spontaneously converting back to the ordered "crystalloid" state. A work group of the inventors was able to demonstrate that the observed confirmation changes in situ can be sufficiently explained by the dependency of the confirmation of crystalline P- proteins from certain divalent cations (Knoblauch, M., Peters, W.S., Ehlers, K. and van Bel, A.J.E.; Reversible calcium-regulated stopcocks in legume sieve tubes. Plant Cell 13,1221-1230 (2001)). The "crystalloid" state is present at Ca^{++} concentrations of significantly less than 1 µM, as can be adjusted with chelating agents, while concentrations in the micromolar range results in transformation into the "dispersed" conformation. With respect to the biological function of the protein bodies, they were given the name "forisomes", derived from "foris" (Latin: door) and "soma" (Greek: σωμα: body).

It was an object of the present invention to isolate the P-proteins, which have been previously observed only in situ, to characterize them, to provide a reproducible method for their preparation, and to check whether, and optionally, for which purpose they could be useful.

In solving this object, the inventors for the first time were successful in isolating forisomes of plants of the legume family (*Fabaceae*). This isolation was found to be extremely difficult. It is indeed possible to push individual protein bodies manually out of the cell and to transfer them onto a microscope slide; however, in doing so, they are destroyed in an uncontrollable way so that the obtained clumps that are more or less amorphous exhibit only minimal reactivity which is expressed in apparent volume changes as a reaction to changes of the calcium concentrations in the medium. To obtain and isolate forisomes from such plants and observe them in intact form was made possible only after many unsuccessful experiments and preliminary trials.
According to the invention it was finally found that it is beneficial to mechanically obtain the phloem as a starting material for isolating the forisomes in such a way that longitudinal sections of the stems or the like of the donor plant is freed of the xylem and, subsequently, the phloem is separated from the cortex and the sclerenchyma. The cells of the phloem are then disintegrated and a suspension of the disintegrated cells is

filtered; the pores of the filter medium preferably have a size which prevents passage of intact cells. After isolation of the fraction containing the forisomes from the filtrate, they can be separated by means of gradient centrifugation from the other components.

For storing the obtained forisomes; a medium containing, for example, 70 % saccharose or 70 % glycerin in V medium (composition see Example 1) (w/v) is suitable; the storability at -20C° is very high; at room temperature it is approximately 3 weeks.

The forisomes isolated in this way, and optionally stored, are completely intact. They have as a whole a pronounced reactivity. In this context it was found that the "dispersed" state is visible and manipulatable in vitro in the same way as the "crystalloid" state. The changes of the optical properties of the protein body upon conversion from "crystalloid" and "dispersed" conformation provides an independent proof for the occurring changes of the inner organization of the protein body.

As has now been determined by means of the undamaged isolated protein bodies, the "dispersion" not only results in a thickening of the protein body in the directions perpendicular to its longitudinal axis but also a contraction along the longitudinal axis (see Fig 1). Triggering of this conformational change can be achieved by providing calcium ions with which the environmental concentration of these ions from less than approximately 30 nM Ca^{2+} (threshold concentration) can be raised to a value that is preferably significantly above this value. The reaction occurs without measurable time delay simultaneously with the change of the calcium concentration; it is complete, and a further increase of the calcium concentration does not affect any change. The thickening is reversible without limitation and practically can be repeated as often as desired (experimentally, at least 50 cycles were checked). The reversal of the conformation change is effected by lowering the calcium ion concentration in the environment of the forisomes to less than 30 nM, for example, by changing the medium or by adding chelating agents for the calcium. The contraction as a result of conformation change is up to approximately 30 %; this is a remarkably high value in

comparison to other actuator proteins. The conformation change takes place within the millisecond range.

The different reaction of completely intact "crystalloids" in comparison to the prior isolated damaged and amorphous clumps indicates that the capability for contraction requires a substantially intact internal organization. However, this does not mean that only original "crystalloids" are able to react. Instead, the inventors were able to demonstrate that even sections which were obtained by smashing frozen protein bodies react in the "customary way" after thawing. Portions of the "crystalloids" react thus autonomously as long as the corresponding inner structure is present. According to the invention, it was determined that this reactivity, based on intact forisomes that are generally approximately 30-40 µm long and approximately 5-10 µm thick, at least up to a comminution in the range which is barely still determinable by light optical microscopy, is maintained. This means that even forisome fragments with dimensions in the range of approximately 1 µm still exhibit the described behavior.

Calcium, similar to in situ, can be functionally replaced by barium and strontium. Magnesium, however, remains without any effect in this connection.

Surprisingly, it was found that crystalline P-protein in vitro also reacts to changes of the pH value. When the pH value increases to above approximately 9.5, in particular, above pH 9.9, a reversible swelling of the protein body occurs. The reaction increases stepwise with regard to its intensity up to a pH value of 10.6. When the pH value drops below a value of 9.5, the swelling disappears. There is no hysteresis. Starting at pH value of 10.9 the forisomes will denature. The swelling induced by basic pH values causes, in contrast to the calcium ion addition, a contraction in the direction of the longitudinal axis of only a few percent so that in the end a volume increase can be observed. It thus differs from the conformation changes effected by cations. In the acidic range the P-proteins are irreversibly denatured at a pH value of approximately 4.5.

The forisomes of the present intention are comprised of two different proteins. This was determined by means of polyacrylamide gel electrophoresis of the protein material that had been isolated, as described above, by means of gradient centrifugation (see Figure 3). The molecular weight of the two proteins that were found in this connection are in the range of approximately 55 to 65 kDa (P1) and 53 to 63 kDA (P2). The aforementioned polyacrylamide gel electrophoresis provides additional protein bands which can be correlated with additional proteins (P3 and P4) which, however, are possibly decomposition products of P1 and P2. This hypothesis is supported by antibody studies. An enzymatic decomposition of the proteins P1 and P2 provides two peptides that have been analyzed by tandem mass spectroscopy (CID-MS/MS). These two peptides have the following structures.

Leu-Gln-Asp-Asn-Pro-Gln-Glu-Val-Ile-Lys (first peptide)
Glu-Gly-Phe-Asp-Ile-Ala-Phe-Lys (second peptide).

According to the invention, forisomes or protein bodies have thus been found in the sieve tubes of the legume family that are comprised of at least two different proteins of comparable size and already in very small sized aggregates or complexes have reversible contracting properties that can be induced, on the one hand, by calcium ions or by other comparable bivalent ions and, on the other hand, also by a change of the pH value. It is remarkable in this connection that the pH value range in which the conformation change occurs is non-physiological and that the two observed conformation changes are different. No ATP as an energy provider is required for the initiation of the conformation change. With regard to contraction in the presence of calcium ions, the protein bodies are similar in certain ways to those of the spasmin of sessile ciliates whose contraction is also calcium-dependent. However, spasmin contracts isotropic and reacts only at calcium concentrations that are 100 times higher.

As a result of the reactivity of very small protein bodies with a diameter of only approximately one micrometer or even less, it can be speculated that during the ontogenesis of the sieve elements in the beginning very small protein bodies with a

correspondingly small diameter have condensed by addition of further subunits to the complex in the micrometer range that can be found today. This would explain why the protein body, despite variable size, is reactive in all stages.

Bio-analog actuators are more and more of interest in bionics in the micro and nano ranges. With their mechanical activity, specific reactivity, longevity and obvious harmlessness for the human organism (legume family members are important foodstuffs), the protein bodies of the present invention have properties as they are demanded for a micro or nano actuator in particular in medical technology. In comparison to other contractile elements of the cyto-skeleton, they have the advantage that their structural coherence is not based on a continuous assembly and disassembly. Other motor proteins of the cyto-skeleton are subject to a continuous turnover which generally significantly limits their longevity in vitro.

The inventors were able to demonstrate that the contraction of the forisomes according to the invention can be converted in a quantifiable manner into force generation. The fact that forisomes pressed onto glass or other surfaces adhere thereto enables, for example, the fixation of individual specimens between two spring arms, for example, made of glass fibers. The adhesive properties of the forisomes on the spring arms can be improved, as needed, by a suitable coating of the material of the spring arms, for example, with poly-L-lysine or adhesives such as, for example, Kwik-Sil (trade name of World Precision Instruments, Sarasota, Florida, U.S.A.). When, by adding calcium ions, the contraction is triggered, each individual protein body produces such a great mechanical force that the spring arms are significantly bent. Such an arrangement is therefore suitable as micro tweezers for manipulating and positioning organic or inorganic structures such as cells, tissue, or molecular bodies. In Figure 4, such tweezers are schematically illustrated: 4A illustrates the open state with relaxed forisome, 4B illustrates the closed state with contracted forisome. The movement of the tweezers can be controlled manually under the light optical microscope or electronically. Since the size of the functioning forisomes is in the range of 1 µm (or even less than that) up to 40 µm, the opening width of the tweezers tip can be adjusted within the

corresponding range. With such tweezers, smallest bodies or substance amounts can be transported in a targeted way and reacted and brought to biological processes at the precise location. As an alternative, the forisomes can be used, for example, as sensor elements for a pH change in the range of approximately 9.5 to 10 and for the increase of calcium ion concentrations in a medium from below to above 30 nM or vice versa and are simultaneously useable as indicator elements: Forisomes can be arranged between two spring arms such that their contraction under the aforementioned conditions of the surroundings effect a contact of the tips of these spring arms. When the tips are electrically conducting, an electrical circuit is closed via these spring arms upon contact of the tips, and the change of the hydrogen or calcium ion concentration in the medium, for example, in an enzymatic process, can be directly detected. Such an element is schematically illustrated in Figure 5: 5A indicates the open electrical circuit with relaxed forisome, 5B shows the closed electrical circuit with contracted forisome.

In the following the invention will be explained with the aid of examples and preliminary and comparative examples in more detail.

Example 1

Purification of forisomes of *Vicia faba.*

Remarks: Vicia faba L. cv Witkiem major (Nunhem Zadenh BV, Haelen, The Netherlands) or other Vicia faba varieties can be grown without problems in a greenhouse.

1. Separation of the root from the shoot at the hypocotyl: the stem of the plants (7-8 weeks old) were separated by a razor blade shortly above the soil line; subsequently, all leaves were removed, and 10 cm of the stem tip cut off.

2. Separation of the cortex inclusive phloem from the xylem cylinder: method for separation of the phloem from the rest of the stem (the intact stem is illustrated in Figure 6A). An effective technique was found in carrying out two oppositely positioned cuts along the longitudinal axis of the plant that cut through the bark to the xylem of the conducting tissue which is annularly arranged (see Figure 6B). Subsequently, the bark was carefully removed along the xylem (Figure 6C.). When doing so, it was observed that the phloem adhered to the inner side of the removed bark and, in this way, could be cleanly removed from the xylem. Special attention had to be directed to the areas of the nodes because here the conducting tissue branches off, and a clean separation is therefore difficult to obtain at these locations. Subsequently, the inner xylem cylinder could be disposed of. Vicia plants in which the secondary thickness growth had not yet begun showed an annular distribution of the sclerenchyma fibers in the cortex upon which the phloem was supported. As a result of the strength of these fibers, they could be easily removed by means of tweezers from the cortex (Figure 6D); the phloem adhered to them.

3. Placement of the cortex into V medium (10 mM EDTA, 10 mM Tris, 100 mM KCl, pH 7.3): a prior incubation for one hour in the V medium facilitated the separation of phloem and sclerenchyma (step 4) and enabled also a regeneration of the forisomes into the crystalline form.

4. Separation of the phloem from the sclerenchyma and cortex: the phloem, after termination of the incubation period, was separated by means of a blunt scalpel from the inner surface of the bark.

5. Pestling the phloem under liquid nitrogen (intermittent sieving is advantageous in order to protect already released forisomes from destruction): the phloem was dried by dabbing with cellulose and was then pestled in a mortar under liquid nitrogen for approximately 10 minutes up to the point of achieving a powder-like state and was then taken up in V medium. The suspension was then filtered

through a tissue sieve and the remaining residue was then thoroughly rinsed several times with 5 ml V medium. The pore size of the tissue sieve was varied and the sizes 100 µm, 80 µm, and 59 µm were tested. The pore size of 59 µm was found to be optimal because the forisomes with a length of approximately 30 µm could fall through without problems even when adhering to other small size material. Moreover, this pore size prevents passage of cells that have not yet been destroyed.

6. The filtrate of step 5 with the forisomes contained therein was centrifuged at 5000 x g at 4°C for 10 minutes. The supernatant was disposed of.

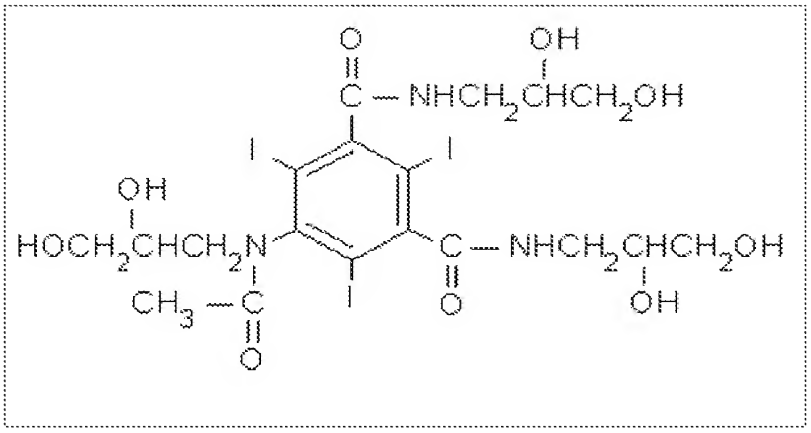
7. The pellet resulting from centrifugation was redissolved in 5 ml V medium and applied onto a Nycodenz gradient (80 - 20 % in V medium): For preparation of gradient centrifugation by means of gradient pouring device a continuous gradient was poured. For this purpose, a column was filled with 15 ml 80 % Nycodenz solution while the other column was filled with 3.75 ml 80 % Nycodenz solution and with 11.25 ml plant suspension - corresponding to a 20 % Nycodenz solution. By means of the gradient pouring device a gradient of 80 % at the bottom up to 20 % at the upper edge of the centrifuge tube was obtained.

8. The Nycodenz gradient according to step 7 was centrifuged for 3 hrs. at 150,000 x g in an ultra centrifuge. By means of this centrifugation a band pattern as illustrated in Figure 7A was obtained. The reference numeral 1 indicates a band of chloroplasts as the uppermost layer. In the middle area identified by reference numeral 2 a wide, relatively sharply discernable band is present which is comprised of membrane residues. At the bottom of the centrifuge tube, identified at 4, a pellet of cell wall debris is found. Approximately at the upper third of the phase between the membrane band and the cell wall pellet a thin band can be identified which is comprised exclusively of forisomes (identified in Figure 7A by reference numeral 3).

9. To the removed fraction (forisome-containing) twice the amount of liquid volume was added and the mixture centrifuged at 5,000 x g for 10 minutes. The supernatant was disposed of.

10. For storing, the forisomes were frozen with 70 % saccharose in V medium (w/v) at -20°C.

Nycodenz has the following formula.



The compound is used as a non-ionic density gradient medium. Reference: Rickwood, D., et al. Anal. Biochem., 123 23 (1982). Beilstein Registry No. 2406632.

Available through Nycomed Pharma AS, Norway.
Synonym: Histodenz (Sigma-Aldrich).

Example 1A

Example 1 was repeated with Vicia plants of the same age whose secondary thickness growth had already started. The separation of cortex and phloem from the xylem cylinder was carried out as follows.

In these plants, the phloem closed to form a cylinder by formation of an interfascicular cambium. The sclerenchyma fibers were also formed stronger so that, after separation of the cortex from xylem, a removal of the sclerenchyma fibers by means of tweezers was difficult to perform and resulted in a high loss of phloem. In these plans, it was much more effective to remove the phloem after termination of the incubation period by means of a blunt scalpel from the inner surface of the bark. This has the advantage that also the sclerenchyma fibers can be separated. Moreover, the yield of phloem is significantly higher than in the Vicia plants without secondary growth.

Example 1B

Example 1 was repeated wherein the gradient centrifugation according to steps 7 and 8 was carried out with a plant suspension to which was added, before pouring the gradient, 0.1 % Triton X100 in order to remove membrane and cell wall proteins and to reduce possibly occurring interactions between the forisomes and the membrane residues. After centrifugation under the same conditions, a band distribution according to Figure 7B was obtained. Even though no chloroplast band could be observed anymore, the forisomes, despite the treatment with Triton, were found primarily within the membrane band (1).

Example 2

Separation of forisomes by means of SDS polyacrylamide gel electrophoresis.

1. The forisomes were centrifuged in V medium for 10 minutes at 5,000 x g.

2. The supernatant was disposed of and the forisomes were taken up in 250 µl 50 mM Tris-HCl buffer pH 6.8.

3. To the forisome solution was added 200 µm 10 % sodium dodecyl sulfate

solution, 25 µl 60 % saccharose solution, and 2.5 µl 2-mercapto ethanol, and the forisome solution was denatured for 5 minutes at 95°C.

4. The mixture was centrifuged for 5 minutes at 12,000 x g.

5. The denatured proteins are separated by means of 10 % SDS polyacrylamide gel according to Laemmli (1970) by electrophoresis.

6. The proteins were made visible subsequently by Coomassie staining. Figure 3 shows the gel with the separated forisome components. The molecular weight is provided in kDa.

Example 3

Determination of the peptide sequences by means of mass spectroscopy.

1. Enzymatic digestion with trypsin.

The protein bands of the SDS PAGE according to Example 2 were cut from the gel and placed into an Eppendorf vessel. After dehydration of the gel pieces by means of acetonitrile, the acetonitrile is removed. Subsequently, the gel pieces were dried in a vacuum centrifuge. Subsequently, 10 mM dithiotreitol (DTT) in 100 mM NH_4HCO_3 were added until all gel pieces are covered completely with the solution. The proteins were reduced for 1 hr. at 56°C. After cooling to room temperature, the DTT solution is replaced by the same volume of 55 mM iodoacetamide in 100 mM NH_4HCO_3. After 45 minutes incubation at room temperature in darkness, the gel pieces are washed with 50-100 µl 100 mM NH_4HCO_3 for 10 minutes, dried with acetonitrile, rehydrogenated with 100 mM NH_4HCO_3 and again dried with the same volume of acetonitrile. The liquid phase is removed and the gel pieces are dried completely in a vacuum centrifuge. Subsequently, to the gel pieces a "digestion buffer" (50 mM NH_4HCO_3, 50 mM

$CaCl_2$, and 12.5 ng/µl trypsin) was added at 0°C. After 45 minutes, the supernatant is removed and replaced with 5-10 µl of a buffer comprised of 50 mM NH_4HCO_3 and 5 mM $CaCl_2$ in order to keep the gel pieces moist during digestion for 12 hours at 37°C. Subsequently, the resulting peptides are extracted with 20 mM NH_4HCO_3 and three-fold extraction with a mixture of 5 % formic acid, 45 desalinated water, and 50 % acetonitrile (each time for 20 minutes) at room temperature, and the combined extracts are dried completely in a vacuum centrifuge.

2. Desalination of the samples.

The sample was taken up in 10 µl 1 % TFA and subsequently desalinated with ZipTipsTM (registered trademark of the Millipore Corporation, Bedford, MA or a subsidiary).
First the equilibration of the tip with 10 µl of a mixture of 50 % water (HPLC grade) and 50 % acetonitrile (HPLC grade) and, subsequently, removal were carried out. This process was repeated once. The tip is then moistened with 10 µl 0.1 % trifluoroacetic acid solution in that this solution is sucked in and released twice.

The peptide mixture from digestion with trypsin is now completely sucked in and then again released. This process is repeated nine times.

The bonded peptides are then freed from salt by washing 10 times with 0.1 % trifluoroacetic acid solution. Finally, the peptides are eluted with 3-10 µl of a mixture of 0.1 % formic acid, 39.9 % water (HPLC grade), and 60 % acetonitrile (HPLC grade). The eluted solution is then again sucked in and released four times.

3 µl of the eluted solution are filled into an nanospray glass capillary. The glass

capillary is fastened in a holder provided for this purpose and the holder is transferred into the nanospray ion source.

Calibration of the mass spectrometer is realized for a mass spectrum with a peptide mixture (1 µmol/l each of angiotensin I, substance P, Glufibrino peptide, renin substrate, ACTH clip 18-39, and bovine insulin) and for MS/MS mode with the peptide Glufibrino peptide (100 nmol/).

3. Recording the mass spectra or the CID-MS/MS spectra

All mass spectra and MS/MS are recorded with a Q-TOF-2 ™ of the company Micromass. For controlling the mass spectrometer and data processing the software programs Masslynx 3.5™ and ProteinLynx 1.0™ are used.
The capillary voltage is between 1.0 - 1.5 kV, the cone voltage is varied within the mass range m/z 400 - 2,500 from 10 eV to 100 eV.

Argon pressure in the collision cell is 10 - 6 mbar for CID-MS/MS (5+1). The recording time is for each scan 2.4 seconds with an interval of 0.1 seconds between each scan.

For de novo sequencing all formed peptide ions of the enzymatically digested protein are selected individually and completely automatically by means of the "collision induced dissociation" (CID) and fragmented (CID-MS/MS). In this context, exclusively 2-, 3-, and 4-times protonated species are selected. Based on the obtained MS/MS spectra sets, sequence information of the corresponding peptides are determined with software support. This sequence information is compared by means of a "blast" search in the Mascot data base in order to find homolog proteins. All MS/MS spectra and the resulting amino acid sequences of the corresponding peptides are evaluated.

Example 4

Induction of contraction of forisomes by calcium ion addition.

Isolated forisomes are transferred into a test chamber of a volume of 1 mL. In this connection, an isolated forisome is pressed by means of a micro injection needle with one end onto the chamber bottom, respectively, in order to prevent drifting when flushing the chamber. Subsequently, by means of an automatic flow-through device alternatingly V medium (10 mM EDTA, 10 mM TRIS pH 7.3, 100 mM KCl) and Ca medium (10 mM $CaCl_2$, 10 mM TRIS-HCl pH 7.3, 100 mM KCl) are applied to the forisome, which results in a conformation change, respectively. The conformation change can be observed microscopically by means of a camera that is mounted on the observing microscope. In Figure 1 the two conformations that the forisome has in the V medium, i.e., in the relaxed state (Figure 1A), and in the Ca medium, i.e., in the contracted state (Figure 1B.), are illustrated. The microscope was set to the direct light interference contrast mode for recording the images. The width of the sections corresponds to 30 µm each.

Example 5

Induction of volume enlargement of forisomes by increasing the pH value.

An isolated forisome is introduced into a test chamber having a volume of 1 ml in which V medium is contained. An isolated forisome is pressed by means of a micro injection needle with one end onto the chamber bottom in order to prevent drifting during flushing of the chamber. Subsequently, starting at a pH value of 7.3, the pH value is increased by means of automatic flow-through devices in steps of 0.3 units; the employed medium remains free of calcium. A first reaction of the forisome can be detected at a pH value of 9.4. The intensity of the reaction increases with increasing pH value up to pH 10.6. The reaction is completely

reversible after introducing V medium of pH 7. Starting at and above a pH value of 10.9 the forisome becomes denatured.

When instead of EDTA/TRIS other buffer media are used that have chelating properties relative to calcium ions, the same reaction course is observed.

In the acidic range, the structure of the forisome will change upon reaching a pH value of 4.9. However, a step-wise increase of the reaction is not observed. Already at a pH value of 4.6 the forisome is irreversibly denatured.

The conformation change can be observed microscopically by means of a camera which is mounted on the observing microscope.

Example 6

Manufacturing micro or nano tweezers for manipulation of individual cells or molecules.

In Figure 8, a tweezers arrangement is illustrated in which two spring arms (for example, glass fibers or glass fiber micro pipettes) are positioned and fastened on a support (for example, by means of an adhesive) at a spacing to one another corresponding to the length of the forisome. In this connection, the forisome can be penetrated at its ends by means of the tips of the spring arms (see Figure 9A). In the case of glass spring arms, the tips which are produced naturally during the production process are used. In the case of other materials, the tips can be produced, for example, by etching. The tweezers are then moved into the vicinity of the object to be gripped such that the object comes to rest between the spring arms in the vicinity of the forisome (see Figure 9A). As an alternative, the forisome can be attached by adhesion on a central location on the spring arms, wherein the spring arms can be coated so as to have increased adhesion should the adhesion of the spring arm on the forisome be insufficient. In this way, glass

surfaces can be coated, for example, with poly-L-lysine or comparable materials. By means of the central positioning of the forisome, an increased spring travel of the arms is obtained in this method (see Figure 10A).

Depending on the material of the spring arms and the different spring constants, their diameter, their length, or the position and/or size of the forisome must be adjusted. In this example, the spring arms are made of borosilicate glass with a diameter of approximately 1-2 µm and a length of approximately 100-500 µm.

The control of the forisome can be achieved in different ways depending on the application. By adding free Ca^{++} to the solution or by pH value changes, a contraction or a volume increase can be triggered. The media for this purpose can be, for example, those mentioned in examples 4 and 5. The contraction by adding Ca^{++} effects closure of the tweezers, as illustrated in Figs. 9B and 10B.

Example 7

Micro or nano switches for detecting pH value and calcium ion concentration changes in a medium.

In a similar way as in Example 6 for tweezers, micro or nano switches can be constructed which are suitable as sensors for indicating a pH value change or a change of calcium iron concentration in the range in which the reversible conformation change of the forisomes takes place. For this purpose, the spring arms are made of corresponding metals, of glass coated with a conducting material, or the like, or electrodes are mounted on the tips (see Figure 11A). The conformation change of the forisomes caused by a corresponding change of the pH value or of the calcium ion concentration in the medium surrounding the forisome moves the contacts together (or opens them) and this results in a noticeable decrease (or increase) of the resistance within the electric circuit; this can be detected with conventional means as a switching step and tapped (see

Figure 11B) The contacts being used are ideally silver electrodes or copper electrodes (depending on the solution). For special fields of application contacts made of other materials are also possible.

Claims:

1. Protein body (forisome) characterized by:
   - its producibility from Fabaceae
   - a reversible, anisotropic contractability with the capability of becoming thicker in the directions perpendicular to a longitudinal axis and shorter along the longitudinal axis for an increase of the surrounding calcium ion concentration past a threshold value of appr. 30 nM, and vice versa, and the capability of becoming thicker in the direction perpendicular to the longitudinal axis without becoming shorter along the longitudinal axis when increasing a surrounding pH value to a value above appr. 9.5, and vice versa.

2. Protein body (forisome) according to claim 1, characterized by a length of approximately 1 µm up to approximately 40 µm and, perpendicularly thereto, with a diameter of approximately 1µm up to approximately 10 µm.

3. Protein body (forisome) according to claim 1 or 2, characterized in that it is comprised of or contains at least 2 different proteins.

4. Protein body (forisome) according to claim 3, characterized in that from the proteins by trypsin digestion the following peptides are detectable:
   Leu-Gln-Asp-Asn-Pro-Gln-Glu-Val-Ile-Lys
   Glu-Gly-Phe-Asp-Ile-Ala-Phe-Lys.

5. Protein body (forisome) according to one of the preceding claims, characterized by a contractibility along the longitudinal axis of up to approximately 30 % with simultaneous expansion perpendicular thereto by approximately 100 %.

6. Peptide or protein comprising or consisting of the following sequence (read from N-terminal to C-terminal):

Leu-Gln-Asp-Asn-Pro-Gln-Glu-Val-Ile-Lys.

7. Peptide or protein comprising or consisting of the following sequence (read from N-terminal to C-terminal):

Glu-Gly-Phe-Asp-Ile-Ala-Phe-Lys.

8. Method for isolating protein bodies (forisomes) according to one of the preceding claims, characterized by the following steps:
   (a) obtaining phloem of a plant of the family Fabaceae;
   (b) destroying the cells of the phloem;
   (c) filtering a suspension of the destroyed cells;
   (d) separating the forisomes from other components of the suspension by means of gradient centrifugation.

9. Method according to claim 8, characterized in that the gradient centrifugation is carried out with a Nycodenz solution, wherein the medium of the suspension according to step (c) contains KCl in a suitable buffer.

10. Method according to claim 8 or 9, characterized in that the plant is from the Fabaceae family of Vicia faba.

11. Use of one or more protein bodies (forisomes) according to one of the claims 1 to 6 as contracting element(s) in micro tweezers or in a detection element for pH changes or changes of the Ca ion concentration.

12. Method for operating micro tweezers characterized in that a forisome of a suitable size according to one of the claims 1 to 6 is arranged stably between two spring arms and subsequently the concentration of calcium ions in the surroundings of the forisome is increased from significantly below 30 nM to significantly above 30

nM.

13. Method for operating a display element for a change of a calcium ion concentration from significantly below 30 nM to significantly above 30 nM or for change of the pH value from below pH 9.4 to greater pH 10.0 in a medium, characterized in that a forisome of a suitable size according to one of the claims 1 to 6 is arranged stably between two spring arms whose tips upon contraction of the protein body can contact one another, wherein the spring arms and their tips are configured such that upon the aforementioned contact an electric circuit is closed and electric current flows, wherein the current flow or the interruption of the electric circuit is detected as a signal that the calcium ion concentration increases significantly above 30 nM or vice versa, or that the pH value rises above pH 10.0 or drops below pH 9.4.

Forisomes, Method for Their Isolation, and Their Use as a Molecular Working Machine

The invention concerns protein bodies (forisome) characterized by their producibility from Fabaceae and a reversible, anisotropic contractability with the capability of becoming thicker in the directions perpendicular to a longitudinal axis and shorter along the longitudinal axis for an increase of the surrounding calcium ion concentration past a threshold value of appr. 30 nM, and vice versa, and the capability of becoming thicker in the direction perpendicular to the longitudinal axis without becoming shorter along the longitudinal axis when increasing a surrounding pH value to a value above appr. 9.5, and vice versa. The invention also concerns a method for isolating these forisomes as well as their use as tweezers or indicating element for calcium ion concentration changes or pH value changes by utilizing their actuator properties.

Figure 1